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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

REMOVABLE MICROFLUIDIC SYSTEM TO DRIVE REAGENTS

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ENCLOSED APPLICATION PARTS (check all that apply)

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☐ Other (specify)
☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

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TITLE OF THE INVENTION

[0001] REMOVABLE MICROFLUIDIC SYSTEM TO DRIVE REAGENTS

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of microfluidics. More specifically, the present invention relates to a microfluidic device that enables to drive liquid phase analytes, molecules or other solutions over microarrays of biomolecules.

BACKGROUND OF THE INVENTION

[0003] Microarrays involve bimolecular interactions where one partner is in solution and the other one is attached to a surface (Howbrook et al., 2003 Drug Discovery Today, 8: 642-651; Kusnezow and Hoheisel, 2003, J. Mol. Recogni. 16: 165-176). For positive interaction to take place, there should be an encounter of the solution phase partner with the surface phase partner. Such an encounter could be driven by several phenomena: diffusion, electrostatic attraction and forced or directed flow. In most conventional microarrays, diffusion is the major driving force. However, this is a slow process requiring between 3 to 16 hours (Maughan et al., 2001, J. Pathol., 195: 3-6). A system using electrostatic attraction demonstrated faster hybridization on arrays made of electrodes (U.S. Patent 6,099,803). However, in these systems low ionic strength solutions must be used. Wang et al demonstrated that dynamic DNA hybridization can be achieved by flowing analytes through a microarray surface using an especially designed array combined with microfluidic circuitry (Wang et al., 2003, Anal. Chem., 75: 1130-40).

[0004] Over the last decade, DNA microarrays have become a powerful tool for genomic and proteomic research. Microarrays allow up to several thousands of nucleic acid probes to be spotted onto very small solid supports (millimeter scale); generally glass slides (Bryant et al., 2004, Lancet Infect. Dis., 4: 100-11; Heller, 2002, Annu. Rev. Biomed. Eng., 4: 129-53; Maughan et al., 2001, J. Pathol., 195: 3-6; Pirrung, 2002, Angew. Chem. Int. Ed., 41: 1276-89).

[0005] Recent efforts were conducted to adapt the microarray technology for rapid identification of biomolecules using signal transduction; the biomolecule binds to a specific probe attached to the solid support (Mikhailovich et al., 2001, J. Clin. Microbiol.,

39: 2531-40; Chizhikov et al., 2001, Appl. Environ. Microbiol., 67: 3258-63; Chizhikov et al., 2002, J. Clin. Microbiol., 40: 2398-407; Wang et al., 2002, FEMS Microbiol. Lett., 213: 175-82; Loy et al., 2002, Appl. Environ. Microbiol., 68: 5064-81; Wilson et al., 2002, Mol. Cell. Probes, 16: 119-27). Such rapid identification is important for diagnostic and forensic purposes, for food and water testing as well as for rapid pathogen detection and identification. Classical DNA microarrays such as Affymetrix's Genechip™ or custom glass-slide technology require hybridization times of up to 18 hours for nucleic acids detection. These methods are thus not fit for rapid molecular testing.

[0006] To speed up the hybridization reaction, several approaches to provide active hybridization systems, or to increase the hybridization dynamics in passive systems have been developed. Electric fields have been used to attract nucleic acid analytes onto capture probes immobilized on electrode surfaces (US patent 6,245,508 ; US patent 6,258,608 ;Weidenhammer et al., 2002, Clin. Chem., 48: 1873-82; Westin et al., 2001, J. Clin. Microbiol., 39: 1097-104). Such a system allows for rapid DNA hybridization (in the order of minutes), but requires expensive hybridization equipment and reader devices.

[0007] Flow-through systems, where targets flow over the probes, increase the probability of interactions between the analyte and the probe. Wang et al disclosed the use of microfluidic circuitries associated with microarrays, and demonstrated that smaller hybridization chambers, in combination with flow-through hybridization, enhanced the hybridization kinetics (Wang et al., 2003, Anal. Chem., 75: 1130-40).

[0008] Microfluidics is an emerging technology allowing to move very small volumes in microscopic tubing adapted for different applications. Channels and chambers are microfabricated in a base of silicon, hard plastic or soft elastomers such as PDMS (Poly-dimethylsiloxane) (Bousse et al., 2000, Annu. Rev. Biophys. Biomol. Struct.; 29: 155-81 ; Anderson et al., 2000, Anal. Chem.; 72: 3158-3164). Fluid propulsion and control valves are designed to allow sequential displacement of liquids into the various segments of the circuits. Numerous microfluidic systems have been set-up for hybridization purposes using different microfluidic technologies (Wang et al., 2003, Anal. Chem., 75: 1130-40; Lenigk et al., 2002, Anal. Biochem., 311: 40-9; Fan et al., 1999, Anal. Chem. 71: 4851-59). However, these technologies are complex, expensive to prototype, and require custom made systems for the arraying of bioprobes and detection of hybridization signals. Noerholm et al developed a microfluidic circuit

engraved in a plastic polymer (Noerholm et al., 2004, LabChip 4: 28-37). The microarray was spotted directly onto the plastic surface of the engraved hybridization chamber. Thus, this system requires a special microarray support, and consequently,cannot be read on commercially available array scanners. Spute and Adey (WO 03/05248 A1) described a three-dimensional fluidic structure for hybridization, but this system requires several layers of microfluidic structures.

[0009] Microarrays constitute a promising technology for the rapid multi-detection of microbial pathogens and their resistance to antimicrobial agents. Currently, hybridization protocols on microarrays are slow, need to be performed by skilled personnel, and are therefore not suited for rapid diagnostic applications such as point of care testing. The merging of microfluidic and microarray technologies provides an elegant solution to automate and speed up microarray hybridization and detection. Such an association has already been described but requires a complex and expensive microfluidic platform.

[0010] There thus remains a need for a rapid, efficient, reliable and low cost method for performing microarray analyses.

[0011] The present invention seeks to meet these and other needs.

[0012] The present description refers to a number of documents, the content of which is herein incorporated by reference in its entirety.

SUMMARY OF THE INVENTION

[0013] The present invention relates to a removable microfluidic system. More precisely, the present invention relates to a microfluidic platform comprising a microarray of bioprobes covered by an elastomeric substrate engrafted with a microfluidic network. Fluids are moved through this network by external forces. The substrate is reversibly bound to the microarray allowing watertightness of the system. The microfluidic substrate can be removed off the microarray allowing it to be analysed externally in a commercial scanner (e.g. scanner based on confocal microscopy).

[0014] The present invention further relates to a device that increases reaction reproducibility, reaction efficiency, and which reduces reaction times and reagent volumes.

[0015] The present invention also relates to a rapid and simple removable fluidic system enabling to drive liquid phase analytes and other solutions over microarrays. In one embodiment, fluids are driven into an elastomeric material engraved with microfluidic circuitry juxtaposed above the microarray. In a preferred embodiment, the microarray is engraved on a standard microscope glass slide. In a more preferred embodiment, the elastomeric material is polymethylsiloxane (PDMS).

[0016] The present invention also relates to a microfluidic system comprising a connected waste reservoir located outside the slide support or any other support, to allow complete drying of the support prior to its analysis. In a particularly preferred embodiment, the waste reservoir is a groove surrounding a disk-shaped slide support in a microfluidic system driven by centrifugal force.

[0017] Further scope and applicability will become apparent from the detailed description given hereinafter. It should be understood however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Having thus generally described the invention, reference will be made to the accompanying drawings, showing by way of illustration only an illustrative embodiment thereof and in which:

[0019] Figure 1 shows a schematic representation of a particular embodiment of the microfluidic system of the present invention. A) Engraved PDMS is applied to a glass slide on which are arrayed nucleic acid capture probes. The glass slide is placed on a compact disc support that can hold five slides. B) PDMS microfluidic unit. The prehybridization hybridization buffer in chamber 2 is released first and flows over the hybridization chamber (chamber 1) where the oligonucleotide capture probes are spotted onto the glass support. The sample in chamber 3, the wash buffer in chamber 4 and the rinsing buffer in chamber 5 then start to flow at a higher angular velocity and are used to wash away the nonspecifically bound targets after the hybridization reaction.

[0020] Figure 2 illustrates a comparison between the sensitivity of labeled oligonucleotide detection in no-flow hybridization (circles) vs flow-through hybridization

(squares) using a complementary 15-mer capture probe.

[0021] Figure 3 illustrates a comparison between the sensitivity of labeled amplicon detection in no flow hybridization (circles) vs flow-through hybridization (squares). The amplicons (368 bp) were generated using a pair of PCR primers targeting Staphylococcus aureus tuf sequences. The S.aureus-specific capture probe was a 20-mer fully complementary to internal sequences of the 368-bp amplicon.

[0022] Figure 4 illustrates flow through hybridization of Cy-labelled tuf gene amplicons by PCR amplification of genomic DNA purified from four staphylococcal species using a microarray of capture probes targeting these four staphylococcal amplicons. Panels: A) Hybridization to the S. aureus amplicons , B) Hybridization to the S. epidermidis amplicons, C) Hybridization to the S. haemolyticus amplicons and D) Hybridization to the S. saprophyticus amplicons.

[0023] Figure 5 illustrates a particular embodiment of a removable microfluidic device for microarray analyses in accordance with the present invention: 1- solid support; 2-microarray ; 3-microarray chamber ; 4-channel ; 5-valve ; 6- reagent reservoir; 7-elastomeric substrate ; 8-airvent.

[0024] Other objects, advantages and features of the present invention will become apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The present invention describes a removable microfluidic system adaptable to arrays printed onto flat surfaces or surrounded by such flat surfaces. The different solutions required for biochemical reactions are driven onto the slide by microfluidic circuitry engraved into an elastomeric substrate juxtaposed onto the surface surrounding the microarray. External forces can be applied to move the fluids; access to various parts of the circuitry is valve-controlled. Non-limiting examples of such external forces are pumps, magnetic, electrokinetic, electro-osmotic and centrifugal. Centrifugal forces can be produced by a motor or a centrifuge and move the fluids into the microfluidic channels and chambers engraved in the surface of the elastomeric substrate positioned above the microarray. The present invention comprises a microfluidic device

having one or more individual chambers connected with one or several reaction chamber(s). The channels and chambers of the microfluidic system of the present invention may access individual spots or group of spots (rows, columns, blocks of spots) of the microarray or the entire microarray. Chamber and channel volumes are generally kept as small as possible to reduce the amount of sample and reagents that must be used.

[0026] The system of the present invention comprises microarray surfaces that are functionalized with an appropriate coating allowing for the binding of probes. The slide format can be adapted to standard microarray equipment frequently encountered in proteomic or genomic labs.

[0027] Each chamber may contain buffers and samples necessary for the chemical reaction(s) to proceed. Small volumes of the fluid sample comprising the biomolecules are forced to flow into the microfluidic circuitry positioned directly above the immobilized probes of the microarrays. The close proximity between the solution phase analytes and the bound probes speeds-up the kinetic interactions, thereby reducing reaction time.

[0028] In a particular embodiment of the present invention, a standard microscope glass-slide is chemically functionalized to covalently bind bioprobes. The microfluidic device may be used to drive fluids over spot-bearing microarrays. The spots may be composed of DNA, RNA, oligonucleotides, proteins, peptides, drugs or combinations thereof, or any binding partner of a substrate present in the test sample. Various reaction steps can be performed with the bound molecules of the microarray including exposure to liquid reagents or reactants, washing reagents, hybridization or detection reagents. The progress or outcome of the reaction can be monitored at each spot of the microarray in order to characterize molecules immobilized on the slide.

[0029] Presently, most custom microarrays are printed onto standard microscope glass slides; this format being required by most of the current commercially available instruments used to scan for detection signals (e.g., fluorescent signal) indicative of positive interactions with particular probes spotted on the slide. The removable microfluidic platform was thus designed to fit standard glass slides. However, any microarray format flat surface (e.g. glass support, plastic support) can be used in accordance with the present invention. Furthermore, such a system may be used in concomitance with independant or integrated microfluidic systems for test sample

preparation (e.g. for nucleic acid extraction) and/or target amplification (e.g. nucleic acid amplification by polymerase chain reaction) for molecular diagnostics. Such a system may also be a micro total analysis system.

[0030] The microfluidic system can be adapted to interface with glass microscope slides and similar planar surfaces. The microfluidic interface system enables the delivery of sample, interacting reagents (e.g., hybridization solutions, binding solutions and the like), wash solutions, and detection reagents to selected positions on the array.

[0031] Grooves or indentations on the surface of the microfluidic system are aligned with spots on the microarray, so that when the microfluidic interface system is sealed onto the microarray surface, the indention and grooves form channel(s), reagent reservoir(s) and/or reaction chamber(s) containing the spots of the microarrays.

[0032] In one particular embodiment, a soft elastomeric material (e.g., PDMS) is selected to make the microchambers and channels of the microfluidic interface system. PDMS-based elastomers are low cost materials which can be easily molded and which seal reversibly with flat and smooth surfaces such as glass.

[0033] In a further particular embodiment, centrifugal forces are used to move the fluids into the microfluidic channels and chambers positioned above the microarray.

[0034] In yet a further particular embodiment, a standard microscope glass slide support is designed to fit into a centrifugation system. The centrifugation system may be a custom device or a classical bench centrifuge. The centrifugation system comprises a step by step motor, controlled by a computer.

[0035] The controlled delivery of fluids to one or more selected regions of the microarray slide may be accomplished by choosing the appropriate size and shape of the channels and chambers of the microfluidic system, and by selecting the optimal centrifugal force and the optimal time over which the centrifugal force will be applied to deliver the fluids over the microarrays.

[0036] In yet a further particular embodiment of the present invention, the microfluidic system is used for the analysis of nucleic acids. The microfluidic system is designed primarily for molecular diagnostic assays on microarrays which typically require rapid, sensitive, automated, high throughput and inexpensive systems.

[0037] As mentioned herein above, the slide can be made of glass, glass being the most commonly used support material for custom microarrays of nucleic acids and proteins. The glass slide is specifically coated to optimize the binding of nucleic acids or nucleic acid analogs (e.g. peptid nucleic acid, locked nucleic acid). Microarrays of nucleic acid probes are printed onto the glass slides using an arrayer positioned to fit directly under the hybridization chamber when the microfluidic circuitry engraved elastomeric material is placed above the slide. Microarrays may include numerous different probes and can be used to perform expression profile experiments. The corresponding hybridization chamber(s) can therefore be designed to accommodate the required volumes, in order to be used as an automated hybridization platform. In a particular embodiment, the array is linear and made-up of 20 spotted probes, and is fitted to be used for diagnostic purposes. The hybridization chamber(s) can also be designed to accommodate smaller volumes allowing flow-through hybridization, thus enhancing the hybridization kinetics. This reduces the hybridization time and/or increases the sensitivity of the reactions required for detection of hybrids.

[0038] In comparison with passive hybridization, the microfluidic device of the present invention allowes for a 10-fold increase in the hybridization kinetics as demonstrated for a 20-mer oligonucleotide as well as for a 368-bp amplicon (see Example 1). Furthermore, it was possible to detect and discriminate 4 clinically relevant Staphylococcus species using a 15-minute hybridization process. This is at least 16 times faster than the times generally required for passive hybridization. The removable microfluidic system of the present invention allows to automate and speed up reaction processes using conventional microarrays and provides for the rapid detection and identification of nucleic acids or other biomolecules present in a sample (proteins, cofactors, drugs and the like). The removable microfluidic system of the present invention can be used in a variety of applications such as in the biomedical field (detection of the presence of pathogens or disease associated markers), in the forensic field (identification of individuals) as well as in basic research. Finally, the removable microfluidic system of the present invention can be applied in any type of microarray analysis.

[0039] In a preferred embodiment, the substrate comprising the microfluidic system of the present invention is a soft elastomeric material capable of reversibly binding to the microarray by Van der Waals forces without the need for any glue or clamp. In a particularly preferred embodiment, the soft substrate is made of PDMS. The

microfluidic circuitry is engraved into the substrate using classical microfabrication technologies such as photolithography and computer numerically controlled (CNC) machining. Various types of valves may be included in the microfluidic circuitry. Valves are designed to control the release of fluids from the different reservoirs. For example, the valves can be electromagnetically actuated microvalves (Canapu et al., 2000, J. Microelectromech. Syst., 9: 181-189), air driven pressure valves (e.g., to control the venting of air in specific regions of the microfluidic circuitry, therefore modulating the backpressure that opposes fluid movements) (Unger et al., 2000, Science, 288: 113), hydrogel valves (Liu et al., 2002, J. MEMS, 11: 45-53), and centrifugal valve ((Madou et al., 2001, Sensor Actuat. A, 91: 301-308).

[0040] The microfluidic flow cell comprises a circuitry engraved into a PDMS substrate. The PDMS substrate was aligned and reversely bound to a microarray printed onto a glass slide by applying soft pressure to form a functional microfluidic unit. After printing the oligonucleotide microarray onto the glass slide using a commercial arrayer, the PDMS microfluidic circuit is superposed onto the glass slide in such a way that the PDMS engraved hybridization chamber is above the microarray. The microfluidic unit (glass slide and PDMS) was introduced into a custom-made plastic disc shape support fixed on the hub of a motor. The disc was rotated to drive sample and buffers directly onto the glass surface using centrifugal forces to move the liquid reagent into the chamber and microfluidic channels (Madou et al., 2001, Sensor Actuat. A, 91: 301-306). At the end of the process, the PDMS fluidic circuits were pealed off the glass slide and the microarray was analysed using commercial instruments. The present system allowed for a comparison between passive hybridization (no flow) and dynamic DNA hybridization (flow-through), generated by the centrifugal forces. The microfluidic device of the present invention was used to discriminate nucleic acid sequences including SNPs (single nucleotide polymorphisms) in a fraction of time required by conventional microarray technology.

[0041] If centrifugal forces are used to drive the fluids in the microfluidic chambers and channels, the valves can be designed to burst at different rotational speeds. The circuitry may comprise a hybridization chamber, a pre-hybridization buffer reservoir, a sample inlet, a washing reservoir, and a rinsing reservoir, all connected together by different sized channels. The different buffers and the sample are forced to flow through the hybridization chamber positioned above the microarray, by varying the rotation speed of the centrifugal system. The architecture of the hybridization chamber

may be adapted to enhance the turbidity of the fluid, thus enhancing the hybridization kinetics.

[0042] The method and device of the present invention uses a microarray support to connect the microfluidic system to the device providing the force to move the fluids. The force can be generated by pneumatic drive, mechanical micropumps, electro-osmosis, electrophoresis, gas-pressure, positive and negative displacement, thermal, electrochemical bubble generation, acoustics, magnetic, DC and AC electrokinetics, and centripetal forces. In a particular embodiment, the support is a disc adaptable to a rotational device providing the centrifugal forces to move the fluids. In a more particular embodiment the support is a disc comprising slots accommodating standard microscope slides. Each slot is placed at the same distance from the disc center, allowing for equal centrifugal forces to be applied to each slotted slide. The disc is designed to be fixed on the hub of a motor. Each slide comprises an aperture, allowing for the easy removal of the slides after centrifugation. In a related embodiment, a furrow is engraved into the support disc to collect the hybridization waste liquid following centrifugation, allowing the slides to dry completely.

[0043] The force-providing device of the present invention can be any device, such as a pump, a heater, a motor, a magnetic device, a mechanical device, or an electrical device. The device providing the centrifugal forces to force the fluids to the microarray support is preferably a motor. The motor may be a step by step motor, or a computer-driven or a programmable, commercially available bench centrifuge.

[0044] Although the microfluidic system of the present invention has been particularly designed to interface with slides bearing microarrays of biomolecules, it may also be used to provide a fluid interfacing with a support bearing various types of molecular probes or samples. The probes or samples could be on bead or particles located on the support. It is to be understood that the application of the present invention is not to be limited to the use with microarray slides.

[0045] The present invention is illustrated in further detail by the following non-limiting examples.

EXAMPLE 1

Removable fluidic system to drive microarray reagents using centrifugal force

Materials and methods

Selected PCR primers and capture probes

[0046] All chemical reagents were obtained from Sigma-Aldrich Co. (St-Louis, MI) and were used without further purification unless otherwise noted. Oligodeoxyribonucleotide capture probes, which were 5'-modified by the addition of two nine carbon spacers and an amino-linker, were synthesized by Biosearch Technologies (Novato,CA). The amino-linker modification permits the covalent attachment of probes onto a functionalized glass surface. Four capture probes were used : Staphylococcus aureus targeting probe (CGTATTATCAAAAGACGAAG), Staphylococcus epidermidis targeting probe (CAIAGCTGAAGTATACGTAT), Staphylococcus haemolyticus targeting probe (CAAAATTTAAAGCAGACGTATA) and Staphylococcus saprophyticus targeting probe (AAAGCGGATGTTTACGTTTT). Primer pairs TstaG422 (AAAGCGGATGTTTACGTTTT) and TstaG765 (TIACCATTTCAGTACCTTCTGGTAA) were used to amplify all staphylococcal species. Used genomic DNAs were purified from strains S. aureus ATCC 43300, S. epidermidis ATCC 14990, S. haemolyticus ATCC 29970 and S. saprophyticus ATCC 35552.

Fabrication of the elastomeric flow cell

[0047] The microfluidic structures were fabricated using PDMS replicating techniques (Duffy et al., 1998, Anal. Chem., 70: 4974-4984). A wide variation of PDMS structures can be molded using microfabricated SU-8 micromolds (Huikko et al., 2003, Lab. Chip, 3: 67-72; Rosqvist et al., 2002, Sensor Actuat. A-Phys., 97: 512-519; Thiebaud et al., 2002, Biosens. Bioelectron., 17: 87-93; Juncker et al., 2001, Micromech. Microeng., 11: 532-541; Jo et al., 2000, Microelectromech., 9: 76-81). A novel 2-level SU-8 process was developed in order to achieve the desired 2-level PDMS fluidic structures that provide sufficient volume for reagent storage while also enabling the proper flow rate for reagent manipulation and for hybridization in the shallow hybridization column.

SU-8 mold fabrication

[0048] SU-8 is a negative tone photoresist that has attracted significant interest for the fabrication, as well as for applications requiring very thick photoresist layers. Due to its excellent UV transparency, standard UV lithography can be used to craft LIGA–like MEMS devices. SU-8 photoresists come in different viscosities: the lower viscosity products are more suited for the fabrication of thin structures (up to 2 μm); the more viscous SU-8 photoresists are better suited for thick layers (mm scale). Two types of the photoresist, SU-8 25 and SU-8 100, available from Microchem Inc. (Newton, MA), were used. SU-8 25 was used for the microchannel structures and SU-8 100 was used for the much larger reagent chambers. In the first step, SU-8 25 was processed on a 15 cm silicon (Si) wafer (Addison Engineering, San Jose, CA) to obtain the structures for the microchannels (25 μm in depth) and the alignment marks for the second SU-8 layer. Subsequently a thick layer (250μm) of SU-8 100 was spin-coated over the substrate on which the molds for the microchannels had been created. This thicker layer was used to define the mold for the much larger reagent reservoirs. Since crosslinked SU-8 photoresists have lower optical transparency than their unexposed surroundings, the alignment marks can be readily observed even when they are completely covered with a thick layer of the unexposed photoresist. In the pattern design, compensations were made for possible alignment errors between the two layers of photoresist. The channels and chambers overlapped in the connection areas to avoid possible disconnections caused by misalignment. Six identical molds were simultaneously fabricated onto the 15 cm Si wafer for faster replication.

Polymerization molding of the flow cell

[0049] Polydimethylsiloxane was purchased from Dow Corning (Midland, MI). The base (Sylguard 184 silicone elastomer) and the curing agent (silicone resin solution) were thoroughly mixed in a weight proportion of 10:1. Low temperature curing (e.g. 65°C) in a convection oven was preferred over high temperature baking due to the thickness of the structures. High temperatures (e.g. 150°C) causes significant thermal stress at the interface between the SU-8 patterns and the Si substrate and which can actually crack the substrate and peel off the SU-8 structures. Leveling of the PDMS on the substrate is required in order to achieve a uniform thickness over all the flow cells. The appropriate combination of the macrostructures of the chambers and microstructures of the channels is important for the performance of the flow cells.

Preparation of glass slides

[0050] All chemical reactions were carried out in polypropylene jars at room temperature unless specified otherwise. The microscope glass slides used (VWR Scientific, West Chester, PA) had a surface of 25 mm x 75 mm. After sonication in deionized water for 1 hour, the slides were sonicated in 40 ml of NaOH (10%) for 1 hour, washed several times with deionized water and dried under a stream of nitrogen. The slides were then sonicated in an aminopropyltrimethoxysilane solution (2 ml water, 38 ml MeOH and 2 ml aminopropyltrimethoxysilane) for 1 hour, washed with methanol, dried and baked at 110°C for 15 minutes. The amine modified slides were activated by overnight sonication in 1,4-dioxane (40 mL) containing 0.32 g (2 mmol) of carbonyldiimidazole as the coupling agent, followed by washing with dioxane and diethyl ether, and drying under a stream of nitrogen.

Microarray production

[0051] The probes were diluted two-fold by the addition of Array-it Microspotting Solution Plus™ (Telechem International, Sunnyvale, CA), to a final concentration of 5 µM. The capture probes were spotted in triplicate, using a VIRTEK SDDC-2™ arrayer (Bio-Rad Laboratories, Hercules, CA) with SMP3 pins (Telechem International). Upon spotting, each spot had a volume of 0.6 nL and a diameter ranging between 140 to 150 µm. After spotting, the slides were dried overnight, washed by immersion in boiling 0.1% Igepal CA-630 for 5 minutes, rinsed in ultra-pure water for 2 minutes, and dried by centrifugation under vacuum for 5 minutes (SpeedVac™ plus; Thermo Savant, Milford, MA). The slides were subsequently stored at room-temperature in a dry, oxygen-free environment.

PCR amplification and amplicon labeling

[0052] Fluorescent dyes were incorporated during assymetrical PCR amplification. Cy-5 dCTP (Amersham Biosciences, Baie d'Urfé, Québec, Canada) was mixed at concentrations of 0.02 µM in a 50 µl PCR mixture containing : 0.05 mM dATP, 0.02 mM dCTP, 0.05 mM dGTP, 0.05 mM dTTP, 5 mM KCl, 1 mM Tris-HCl (pH 9), 0.01% Triton X-100, 2.5 mM MgCl2, 0.5 Unit of Taq DNA polymerase (Promega, Madison, Wisconsin), 0.2 µM of primerTstaG765, 0.005 µM of primer TstagG422 and 1 ng of purified staphylococcal genomic DNA. Thermal cycling for PCR amplification (180 s at 94°C followed by 40 cycles of 5 s at 95°C, 30 s at 55°C, and 30 s at 72°C) was

carried out on a PTC-200 DNA Engine Thermocycler™ (MJ Research, Reno, NV).

DNA microarray hybridization and data acquisition

[0053] Cy-dUTP labeled PCR amplicons were denatured at 95°C for 5 minutes. Denatured labeled amplicons (5 µl) were mixed with hybridization buffer (15 µl) (8X SSPE, 0.04% PVP and 40% formamide).

[0054] Passive hybridization was performed in a 20 µl Hybri-well™ self-sticking hybridization chambers (15 mm x 13 mm) (Sigma-Aldrich). Hybridization buffer containing the labeled sample was introduced in the chambers and hybridization was conducted for 5 minutes at room temperature. After hybridization, the microarrays were washed at room temperature (5 minutes) with 2X SSPE containing 0.1% SDS and rinsed once (5 minutes) with 2X SSPE at room temperature. The microarrays were dried by centrifugation at 1348 x g for 3 minutes.

[0055] Flow-through hybridization was performed in the flow-cell as described above. A hybridization unit consisting of a glass slide and flow-cell was placed onto a home made plastic disc support, and the support fixed to the hub of a step by step motor controlled by a computer. The labeled sample was prepared the same way as for a passive hybridization. Sample (2µl) and washing and rinsing buffer (10 µl) were loaded onto the microfluidic unit just before spinning the disc. The disc was spun at different speeds in order to sequentially burst the centrifugal valves and allow the pre-hybridization buffer, sample, washing and rinsing buffer to flow-through a 140 nl hybridization chamber respectively. The disc was subsequently spun at 1000 RPM for 1 minute to dry the slide.

[0056] Slides were scanned using a ScanArray 4000XL™ (Packard Bioscience Biochip Technologies; Billerica, MA) and fluorescent signals were analyzed using its QuantArray™ software.

Results

Assembly of the microfluidic unit

[0057] The assembled microfluidic unit is shown in Figure 1. The flow cell is aligned with, and adhered to, the glass slide to form a DNA hybridization detection unit, up to 6 of which can be mounted into the CD platform (Figure 1(a)). The design of the

microfluidic network and the microarray layout is such that a hybridization chamber is positioned right above the oligonucleotide capture probes spotted onto the glass slide when the two parts are put together. The reagents are positioned to be sequentially pumped through the hybridization chamber by centrifugal force beginning with chamber 1. This flow sequence is achieved by optimizing the balance between the capillary force and centrifugal pressure (Madou et al., 2001, Sensor Actuat. A, 91: 301-306) (Figure 1b). The pre-hybridization buffer (chamber 2) is released first and flows over the 140 nl hybridization chamber (chamber 1) where the oligonucleotide capture probes are spotted onto the glass support. The wash buffer (chamber 4) and the rinsing buffer (chamber 5) flow at a higher angular velocity and are used to wash the nonspecifically bound targets following the hybridization process.

Flow-through in small volume vs passive hybridization in larger volume

[0058] Cy3-labeled 20-mer oligonucleotides were hybridized both in a passive way using a standard commercially available hybridization chamber of 20µl, and with the flow-through method using the microfluidic platform as described hereinabove (140 nl of hybridization chamber). For passive hybridization, 20µl of different concentrations (i.e. 0.025 nM, 0.125 nM, 0.25 nM, 1.25 nM and 2.5 nM) of Cy3-labeled oligonucleotides were hybridized to their complementary probes, spotted onto a glass microarray using Hybri-well™ self-sticking hybridization chambers (Sigma-Aldrich). Following hybridization at room temperature for 5 minutes, the slides were washed and rinsed. For flow-through hybridization, 2 µl of 0.025nm, 0.125nM, 0.25nM, 1.25 nM or 2.5 nM of Cy3-labeled oligonucleotide were hybridized to their complementary probes as described for the passive method. Samples of the different concentrations of oligonucleotides (2 µl) were loaded into the sample inlet of the microfluidic unit. Prehybridization buffer, sample, washing buffer and rinsing buffer were loaded respectively into chambers 2, 3, 4 and 5 of the hybridization unit shown in Figure 1b. Loading of the reagents was performed immediately before spinning the disc platform to avoid reagent evaporation. A spin speed was selected so as to obtain a sample flow rate of about 400 nl/min in the hybridization chamber, which corresponds to a hybridization time of 5 minutes (identical to the hybridization time used in the passive hybridization experiments). Following the hybridization step, the spin speed of the platform was increased in order to sequentially burst the centrifugation valves, releasing respectively into the hybridization chamber 10 µl of washing buffer and 10 µl of rinsing buffer, both of

gene expression. Thousands of genes are routinely studied for their expression using the microarray technology. Several groups have attempted to adapt this technology to the rapid detection of microbial targets for diagnostic purposes (Mikhailovich et al., 2001, J. Clin. Microbiol., 39: 2531-40; Westin et al., 2001, J. Clin. Microbiol., 39: 1097-104; Wang et al., 2003, Anal. Chem., 75: 1130-40; Bavykin et al., 2001, Appl. Environ. Microbiol., 67: 922-8; Bekal et al., 2003, J. Clin. Microbiol., 41: 2113-25). Even though such systems most often require highly advanced biochips, the detection performance lacks both sensitivity and specificity (Lenigk et al., 2002, Anal. Biochem., 311: 40-9; Wang et al., 2003, Anal. Chem., 75: 1130-40). As demonstrated hereinabove, very small amounts of liquid can be precisely and directly moved onto a glass slide surface from buffer chamber(s) to hybridization chamber(s) using a microfluidic elastomeric flow-cell juxtaposed above the slide. This technology allows to dramatically reduce the volumes of reagents required during microarray hybridization. For identical concentrations of a 20-mer oligonucleotide or 368-bp amplicon, the flow-though hybridization method gave signals which were of an order of magnitude higher than those obtain with passive hybridization (Figure 2). Theses results confirm previous observations, obtained with a more complex microfluidic device (Wang et al., 2003, Anal. Chem., 75: 1130-40). The capture probes and buffer compositions were designed in order to achieve hybridization at room temperature, thereby reducing the complexity of the device.

[0062] The system of the present invention is specific enough to discriminate SNP at room temperature using a hybridization period of less than 10 minutes. The results of the discrimination specificity for 4 staphylococcal species (hybridization period of 5 minutes) are shown in Figure 4 . Other probes have at least 3 distinct nucleotides as compared with others staphylococcal amplicons. In addition to being specific, the present system is also sensitive. It is possible to generate a high hybridization signal using only 10% of an amplified PCR reaction mixture containing the equivalent of 1000 copies of staphylococcal genome as starting material. This result is at least 10 times more sensitive than those obtained by other groups using more complex microfluidic devices (Westin et al., 2001, J. Clin. Microbiol., 39: 1097-104). It is worth noting that the PCR products are not purified prior to their addition to the hybridization buffer.

[0063] Overall, the present example describes an affordable, easy to use, automated and rapid custom microarray hybridization microfluidic platform. This microfluidic platform uses standard glass slides totally compatible with commercial arrayers and scanners. In this system the classical hybridization chamber or coverslip is

which flowed through the hybridization chamber with an average flow rate of 2 µl/min resulting in a total time of about 15 minutes for the entire hybridization process, including a 30 second drying step (high spin speed). The PDMS microfluidic flow cells were pealed off. The hybridized microarrays, following the passive or the flow through method, were scanned using a Scanarray 40000XL™. The fluorescence intensity was spotted against concentration of oligonucleotide (Figure 2). It was observed that flow through hybridization in a 140 nl chamber was more sensitive than a passive hybridization in a larger volume chamber (i.e 20 µl) (Figure 2). The passive and flow through hybridizations were also performed using a 368-bp Cy-labeled amplicon that is derived from tuf gene sequences. The results of these experiments (Figure 3) show that flow through hybridization is more sensitive than passive hybridization as observed with a complementary oligonucleotide (Figure 2).

Sensitivity of the microfluidic platform

[0059] In all the experiments described above, the standard procedure was to perform PCR amplification using 1 ng of genomic DNA from different strains of staphylococci. Approximately, 1% of the amplified PCR reaction mixture was used for each hybridization. To evaluate the minimal quantity of bacterial genome needed to have a clear and unambiguous signal using the microfluidic platform, hybridization of PCR amplicons amplified from the equivalent of 10, 100, 1000 or 10000 genome copies was performed. It was found that the equivalent of as little as 1000 genome copies of starting material was enough to discriminate each of the four staphylococcal amplicons.

Detection and identification of 4 clinically important Staphylococcus species

[0060] Universal primers targeting the conserved area of the tuf gene were used to amplify a 368-bp fragment from S. aureus, S. epidermidis, S. haemolyticus and S. saprophyticus purified genomic DNA. Microarrays of specific capture probes, printed in duplicate, targeting these 4 staphylococcal species were prepared and hybridized with the 4 different staphylococcal amplicons. The results demonstrate that it was possible to detect and discriminate the four different staphylococcal tuf amplicons without any ambiguity. It is worth noting that there is a difference of a single nucleotide mismatch (SNP) between the S. epidermidis specific probe and the S. aureus amplicon sequence, showing that this system is able to distinguish SNP in only 5 minutes of hybridization.

[0061] In the genomic field, microarrays have become the standard for profiling

replaced by a low cost elastomeric material engrafted with a microfluidic network. This elastomeric material sticks reversibly without any glue or chemical reaction to the glass slide, forming the microfluidic unit. Placed in a plastic compact disc like support, the microfluidic units are spun at different speeds to allow fluids to move. Using the present system it was demonstrated that it is possible to detect and discriminate tuf sequences from 4 different Staphylococcus species using a rapid hybridization protocol of 15 minutes.

[0064] Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A removable microfluidic system interfacing with a support including a microarray, said microfluidic system comprising a microfluidic network engraved into a substrate.

2. A removable microfluidic system as defined in claim 1, wherein said substrate is an elastomeric material.

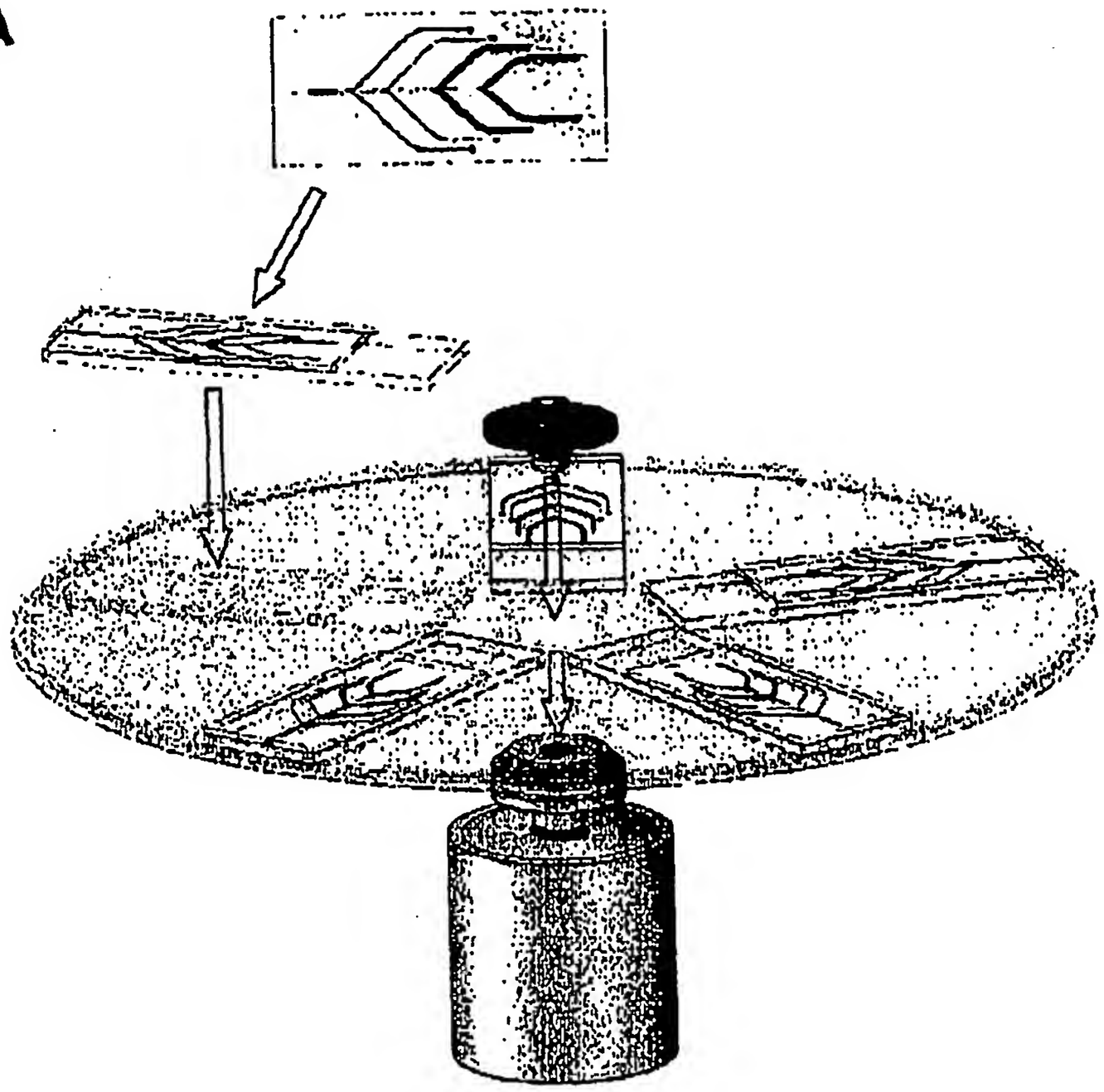
3. A removable microfluidic system as defined in claim 2, wherein said elastomeric material is PDMS.

4. A removable microfluidic system as defined in claim 1, wherein said support is a glass slide.

ABSTRACT OF THE DISCLOSURE

The present invention relates generally to the field of microarray technology. More specifically, the present invention relates to a removable microfluidic device that enables to drive liquid phase analytes, biomolecules or other solutions over microarrays or related planar surfaces, by using external forces such as centrifugal forces. The microarray is then analysed using a reading device.

A



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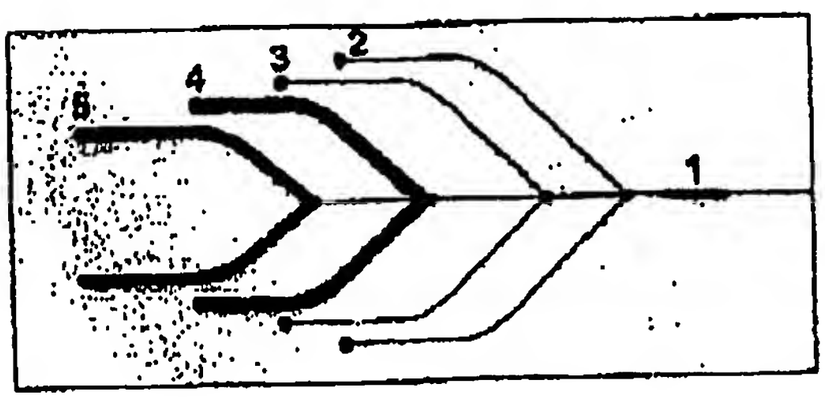


Figure 1

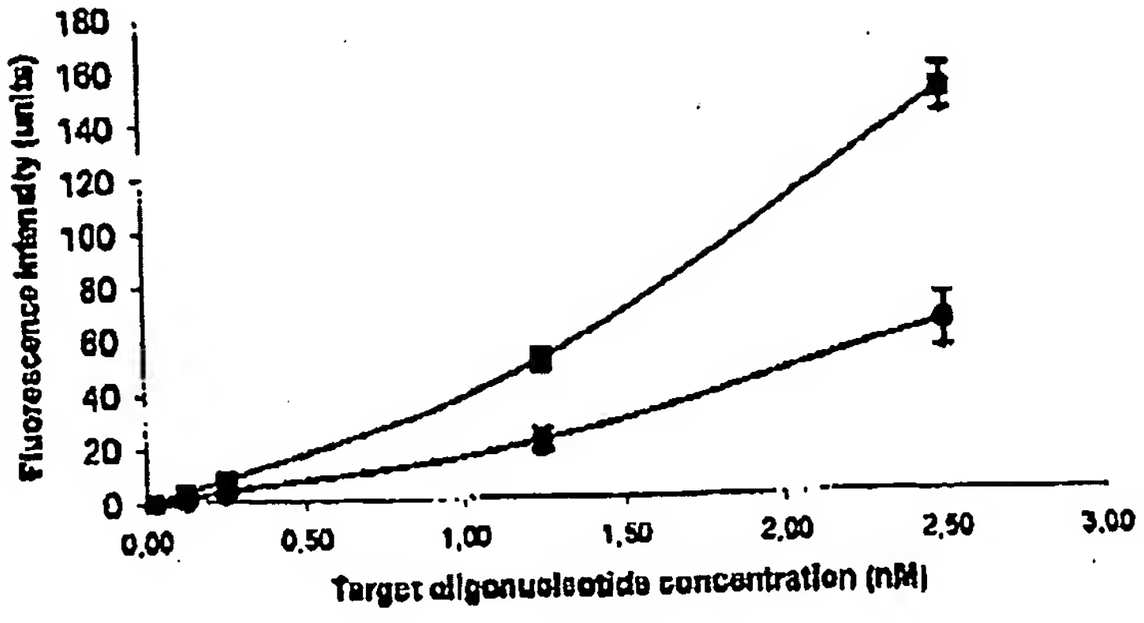


Figure 2

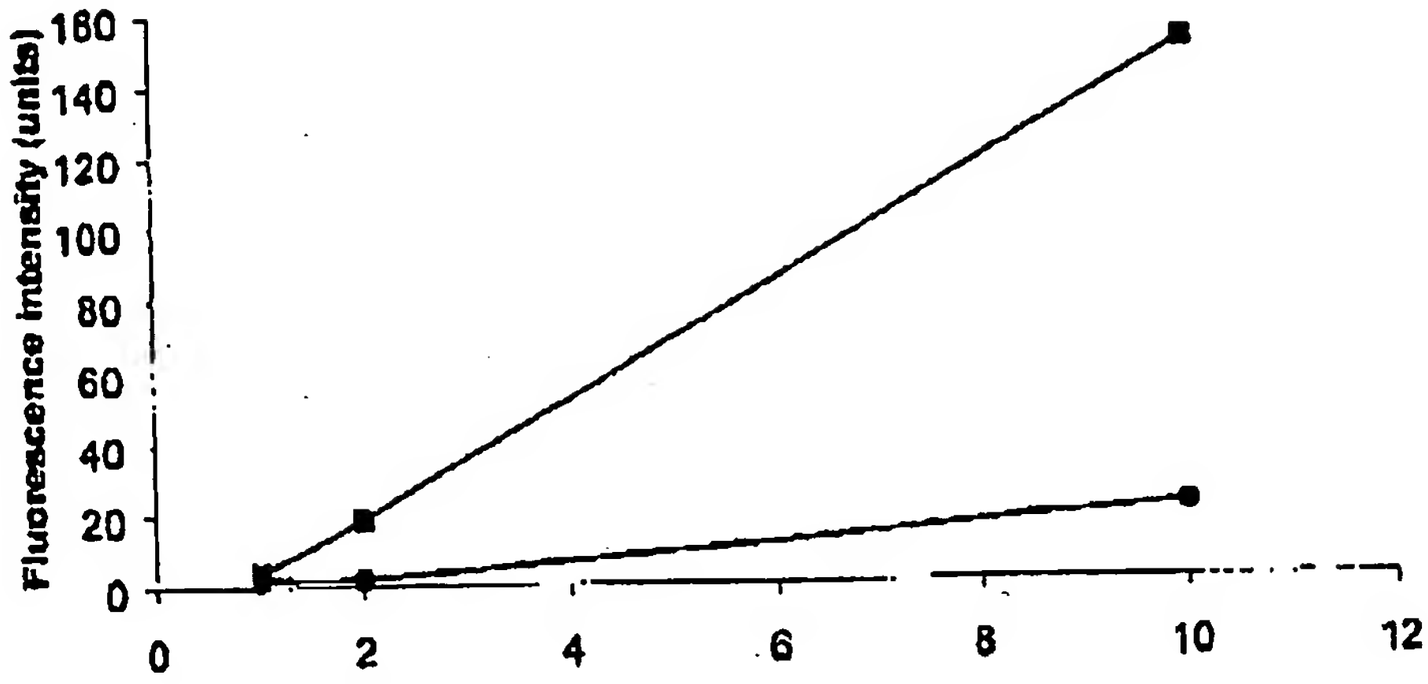


Figure 3

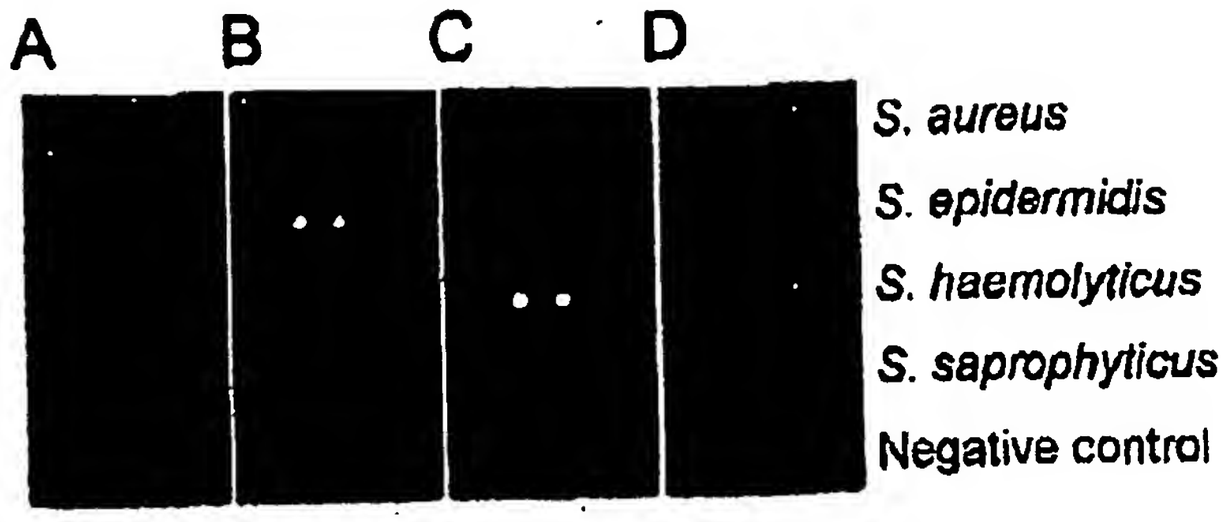
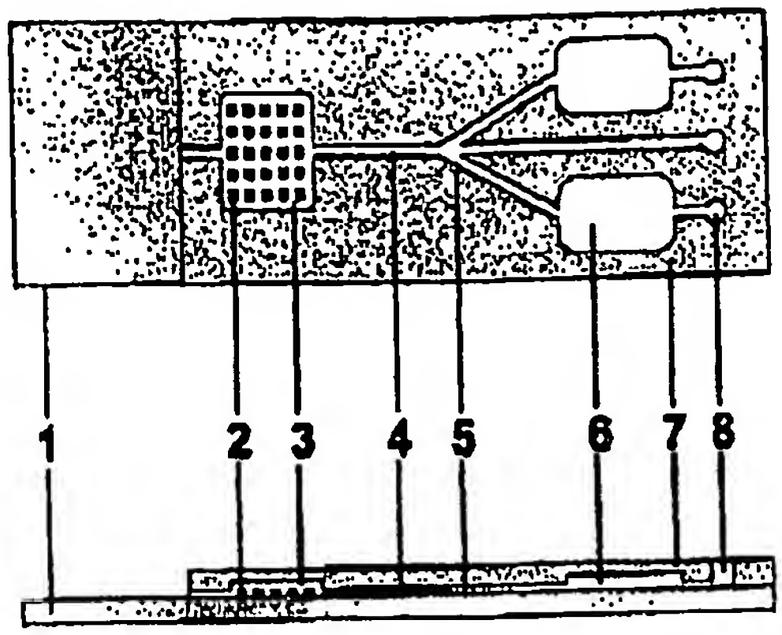


Figure 4



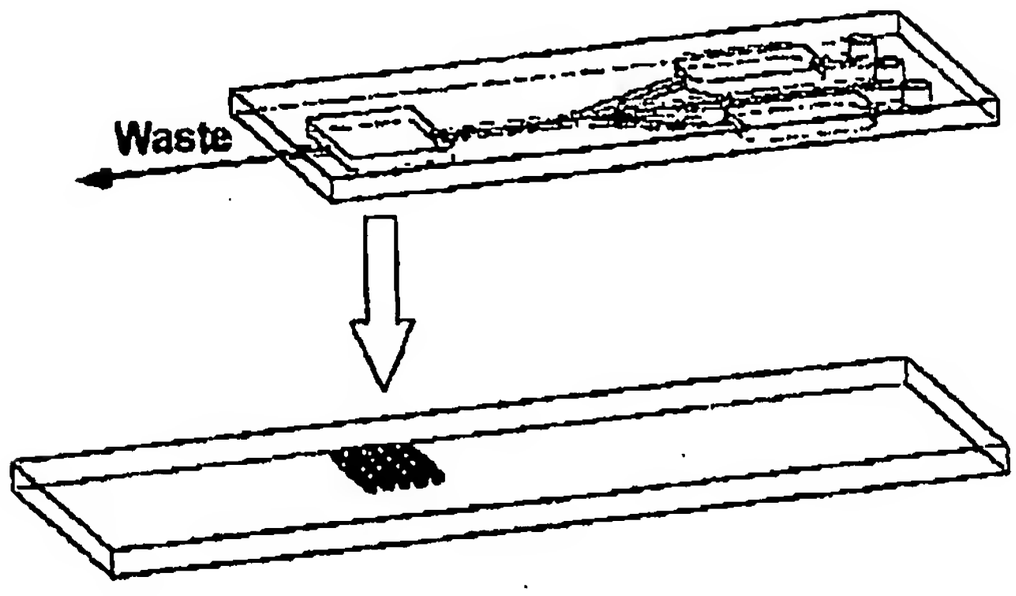


Figure 5